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<b>14. ABSTRACT</b>  We have an Interdisciplinary project testing approximately 600 samples each in two novel methods(CyTOF-phosphoflow and HLA Typing) to help us understand the underlying genetic and immune system roles between CFS cases and controls.  For the CyTOF Phospho-flow testing, there will be continuous testing until we finish all the samples that need to be completed. We have also taken a preliminary look at the data to get a sense of the output and the possible statistical analysis that could be performed once all the data is in. We will continue to work on the best statistical methods as more data becomes available.  For the HLA typing testing, we are taking the necessary steps to complete the testing and look forward to studying the results. We have finished the DNA isolation and anticipate the HLA testing to be completed this upcoming year. We want to interrogate the different HLA types and take into account possible genetic and environmental exposures. The goal is to see if genetic make-up (or specific antigen) at the HLA level that makes you more susceptible to have Chronic Fatigue Syndrome (CFS) or any differences between the cases and controls. In order to extract the best information on these patients, more HLA patient data needs to be included.			

**ABSTRACT(CONTINUED)**

In summary, we are aware of the challenges for the last year of testing and have a plan in place to meet the goals of this research study. Again, our primary goal is to help validate the model of CFS as a heterogeneous illness that results from a complex interaction of genetic, immune, and infectious factors. The data gained from these 2 novel tests (CyTOF-phosphoflow and HLA Typing) will give us new data on a large cohort for analysis. The discussions from our interdisciplinary team have helped us identify process improvements and prepare for data analysis. We are looking forward to the completion of testing so that we can execute these analytical plans to see if we can better understand the underlying cause of CFS.

**15. SUBJECT TERMS**

CyTOF, human leukocyte antigens (HLA) types, Chronic Fatigue Syndrome(CFS), novel testing, autoimmune disease, dynamic range, analytes, phospho-flow, flow cytometry, pico-green, quality control, Chronic Fatigue Immune Dysfunction, genetic, immune, infectious disease

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## **INTRODUCTION:**

We are excited to complete the HLA and CyTOF testing in the final year as well as to see the information yielded by the results. It will be interesting to see the roles that genetics and the immune system play as well as the differences between CFS cases and Controls. All team members are aware of the goals for this DOD Grant and we look forward to completing all the tasks as needed in order to complete the project together.

For the CyTOF Phospho-flow testing, we have continued to progress, troubleshoot, and identify the best processes to move forward. We have taken preliminary looks at the data to get a sense of the output and the possible statistical analysis that could be performed once all the data is in. We will continue to work on the best statistical methods as more data becomes available. We have also ramped up the throughput to complete testing by July 2015.

For the HLA typing testing, we are pleased with the progress and look forward to studying the results. There have been studies to show that HLA classes may be implicated in certain Autoimmune Diseases. These HLA markers may contribute to the genetic susceptibility for autoimmunity. We want to interrogate these specific sites and take into account possible genetic and environmental exposures. The main goal for our analysis is to see if there is a genetic make-up (or specific antigen) at the HLA level that makes you more susceptible to have Chronic Fatigue Syndrome (CFS) or any differences between the cases and controls. We feel that we will be able to answer a lot of questions once we receive the entire collection of data that is being pursued.

## **BODY (CyTOF):**

We are continuing to test the CyTOF Phospho-flow assay and have an idea of the data to expect from these samples so far. Over 200 of the 600 samples have been processed to date.

We have stepped up our throughput so that we will have completed about 300 samples by September, and all 600 by July 2015.

We have also taken a preliminary look at the interim data to start the plan for analysis once all the samples have been tested. We have met with the statisticians and scientists to prepare for how we will pursue the analysis by conducting multiple meetings with Dr. Maecker and Rosemary as well as the statisticians to look at preliminary data. We have investigated the batch-to-batch variability in depth and have switched from automation to manual washing of the cells. There were some issues with the automation, so we decided it would be better to manually wash as it was yielding possibly better results. There was a breakdown in the automation equipment during the processing of one of the batches, which necessitated manual washing of the cells. Since then, all subsequent batches have been processed manually. We think that the data suggest that this is working better than the fully automated method used. There is greater dynamic range for many analytes and more cells per sample when washing manually. This has proven to take more manual efforts and time, but our team felt this would be the best method moving forward for the integrity of the data.

The interim analysis suggests that a few readouts may show group-level differences between cases and controls. For example, STAT1 response to IFN $\alpha$  stimulation in naive B cells appears consistently lower in cases versus controls across the first five batches (Appendix A). This analysis used arcsinh transformed differences between unstimulated and stimulated samples, which partially corrects for batch effects in the data. The formula for this transformation used was:  $\text{asinh}(X/5) - \text{asinh}(Y/5)$ , where  $X = \text{median}(\text{stimulated})$ ,  $Y = \text{median}(\text{US})$ . 5 is a cofactor that has been found most optimal for CyTOF data. In addition to this preliminary analysis, we have looked at median values as well. We may also perform global normalization per batch, rather than normalization to the control sample. Finally, we would like to look for any stimulation conditions that yield no significant differences between cases and controls, for any cell types (either looking at asinh difference or medians). These stimulators would be candidates for removal from the panel. We will continue to examine whether additional normalization for batch effects is necessary, and will update these analyses as we have more data.

As you can see, we have been working towards the testing as well as the statistics and science for this testing. The lab plans to ramp up testing for this last year of this DOD grant. The CyTOF Phosphoflow assay panel (Appendix B) shows the complexity of information for each sample. For each patient sample, there are a number of combinations being tested of different cell subsets, phosphoepitopes, and stim conditions. By using this novel approach, looking at the mass cytometry we are able to gain a lot more info than traditional testing. We have streamlined the workflow in order to sufficiently test all the parameters desired. We have also created a data template so that the data is being delivered in a consistent manner for statistical analysis as well as uploading the data into a Stanford Data Miner (SDM) platform that will be helpful to make it easier to look at the data. We look forward to the completion of the testing in the final year of testing.

#### **BODY (HLA Typing):**

For the HLA testing, we have continued to progress in the completion of testing. We have finished the DNA isolation for all of the ~600 samples to be tested. All the isolated DNA samples have been delivered to the Genome lab and are ready for testing. Over 360 of 600 samples have been processed to date. We anticipate the rest of the samples will be done with testing by next year, with plates 1-4 being done and in the LIMS. These 4 out of the 7 plates have results and the final 3 plates are in the process of being completed. All PCR were done as in Dough Levinson (i.e. A, B, C, DPA, DPB, DRB with old conditions and DQA, DQB with modified dNTPs and new primers). They have been pico-greened and will be normalized as well.

Once the data results for the 4 plates were received, we took a look at the data to ensure accuracy. Quality Control was performed on the HLA preliminary results. We have verified the HLA sample data versus the batch list to ensure that all the samples were tested accordingly. This has been done for all 4 plates that have already been tested, for which results have been given. We are working to get the dataset cleaned and streamlined for analysis.

We have also taken a preliminary look at the data for these 1<sup>st</sup> 4 plates as well. The main goal for our analysis is to see if there is a genetic make-up (or specific antigen) at the HLA level that makes you more susceptible to have Chronic Fatigue Syndrome (CFS) or any differences between the cases and controls. There have been studies done to show a possible association between HLA class II antigens and Chronic Fatigue Immune Dysfunction and the methods for this HLA novel testing are using the highest resolution for genome, so we will obtain a lot more useful information. For the interim analysis it looks like the 2 alleles B 0702 and C 0702 may create greater signal when combined, so this is something we can look into once all the data has been received as well. We have also provided the following clinical features to help with the breadth of analysis. Some highlighted clinical features are:

- Gender/Sex
- Case/Control Status
- Fatigue Severity Scale (FSS) Average (A questionnaire to evaluate fatigue)
- MFI-20 Score (A questionnaire to evaluate fatigue to capture ‘severity’ of the disease)
- Viral Onset (Whether CFS pts felt their fatigue started through some sort of viral onset)
- Fatigue Onset (Used by our stats team to calculate duration of illness for CFS patients)
- Self-reported physical and cognitive functioning (out of 100%)

Hopefully these clinical data will help to further characterize the patient population and supplement the analysis for the HLA data. Also, all of the patient samples are from the Bay Area, so this will take into account hopefully the same environmental exposures. Additionally, we will look into the races of these patients, but more patients are needed to be added to the data sets. With a larger sample size, populations tend to clump together. We hope once all the testing is completed, it will give us a better picture of the 2 distinct patient populations (CFS vs Control). The clinical data will also help us subgroup the complexity of CFS as well.



## **KEY RESEARCH ACCOMPLISHMENTS:**

- Year 2 has allowed us to review some of the data output to better prepare for statistical analysis as well as to continue with the completion of CyTOF and HLA Typing testing

### **For CyTOF, there have been over 200 samples tested.**

- Stepped up our throughput to complete testing by July 2015
- Taken a preliminary look at the interim data to start the plan for analysis
- Preliminary data analysis using the arcsinh transformation as well as median values
- Multiple meetings with Statisticians and Scientists to visualize the data and understand the processes to handle the complexity of the data
- Investigated batch-to-batch variability
- Moving forward with manually washing the cells to yield better results
- Created data templates in order to obtain reproducible data formatting

### **For HLA Typing, there have been over 360 samples tested.**

- Completed the DNA isolation for all of the samples to be tested.
- Received data from 4 out of the 7 plates
- PCR has been done and have been pico-greened and normalized
- QC of the data received to ensure accuracy of what was tested
- Working to get the dataset cleaned and streamlined for analysis.
- Provided Clinical Characterization of patients to enhance the analysis

## **REPORTABLE OUTCOMES:**

There are no current reportable outcomes as we are still completing the testing phase.

## **CONCLUSIONS:**

As we mentioned during our first annual report, this research will help validate the model of CFS as a heterogeneous illness that results from a complex interaction of genetic, immune, and infectious factors. We have discovered first hand at some of the complexities of the data, but our team is up for the challenge.

For the CyTOF testing, there will be continuous testing until we finish all the samples that need to be completed. Our team has reserved 3 full days of CyTOF run time per week until all ~600 samples are done. This includes staining batches of 9 samples at a time, plus our internal control. These 10 total samples comprise of a total of 80 stim conditions. The technology has given us the opportunity to look at high throughput data with a volume of information, but believe we have a team that is committed to completing this project on time.

For the HLA testing, we have progressed and are on track to finish the testing. We have completed the DNA isolation for the samples and anticipate the processing/testing to be completed this next year. There are 7 plates of sample DNA and 4 of these plates have been tested with HLA data results. Once we received the data for these first 4 plates of approximately 360 patients, we took a look at the data to ensure accuracy as well as to begin with preliminary analysis ideas. We also have included some important clinical features that will help with analysis. In order to have a clearer picture of the data, more HLA patient data needs to be included.

We continue to have meetings to move this project forward. The discussions from all parties have helped identify process improvements and prepare for data analysis. We are looking forward to the completion of testing so that we can execute these analytical plans to see if we can better understand the underlying cause of CFS. This last year has afforded us to progress with the testing phase with a plan in place to continue with the testing for the next year. Once all the data is collected, we are very interested to see the results and let the data tell us what genetic and immune factors are involved in CFS.

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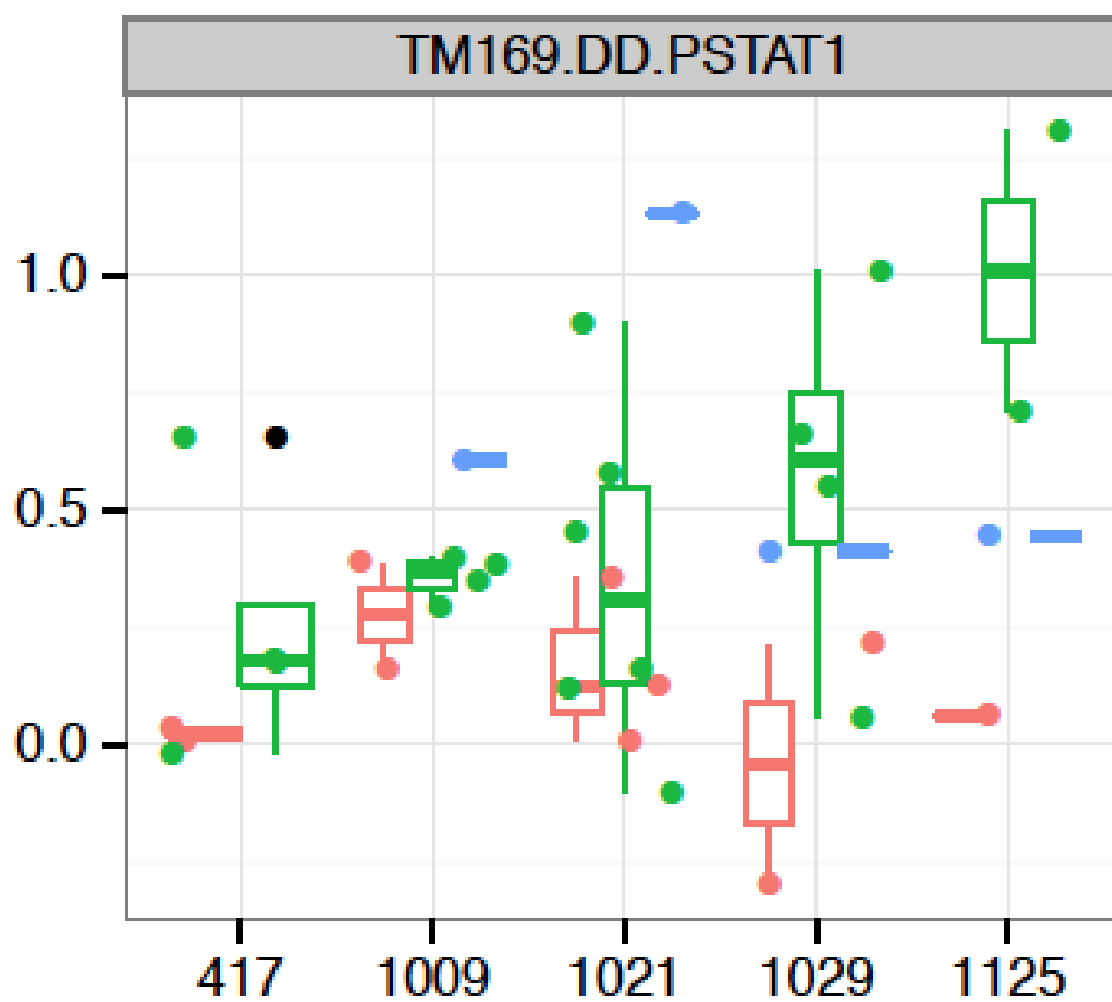
## APPENDICES:

**Appendix A:** Example of STAT1 response to IFN $\alpha$  stimulation in naive B cells

**Appendix B:** CyTOF Phosphoflow Assay used for testing

**Appendix C:** HLA Results Data Example

**Appendix A:** Example of STAT1 response to IFNa stimulation in naive B cells



## Appendix B: CyTOF Phosphoflow Assay used for testing

CyTOF Phospho-flow Assay Panel

Cell subset	Phosphoepitopes	Stim Conditions
Basophils	cells/intact/Non Basophils/Lymphocytes/CD3+/CD8+T/Naive, <b>Freq. of Parent</b>	Unstimulated (US)
CD4+T	cells/intact/Non Basophils/Lymphocytes/CD3+/CD8+T/Naive,Median,(Dy162)Dd, <b>pERK</b>	IFNa
CD4+Effector	cells/intact/Non Basophils/Lymphocytes/CD3+/CD8+T/Naive,Median,(Dy163)Dd, <b>IkBtot</b>	IL6
CD4+Naïve	cells/intact/Non Basophils/Lymphocytes/CD3+/CD8+T/Naive,Median,(Gd156)Dd, <b>pp38</b>	IL7
CD4+ Central Memory	cells/intact/Non Basophils/Lymphocytes/CD3+/CD8+T/Naive,Median,(Gd158)Dd, <b>pSTAT3</b>	IL10
CD4+Effector Memory	cells/intact/Non Basophils/Lymphocytes/CD3+/CD8+T/Naive,Median,(Ho165)Dd, <b>pS6</b>	IL21
CD4+ HLA-Dr+CD38+	cells/intact/Non Basophils/Lymphocytes/CD3+/CD8+T/Naive,Median,(Tm169)Dd, <b>pSTAT1</b>	LPS
Treg	cells/intact/Non Basophils/Lymphocytes/CD3+/CD8+T/Naive,Median,(Yb172)Dd, <b>pSTAT5</b>	PMA
CD4-CD8-	cells/intact/Non Basophils/Lymphocytes/CD3+/CD8+T/Naive,Median,(Yb173)Dd, <b>pPLCg2</b>	
CD8+T		
CD8+CM		
CD8+Effector		
CD8+Effector Memory		
CD8+HLA-Dr+CD38+		
CD8+Naïve		
B cells		
B Switched Memory		
IgD+ Memory		
Naïve B		
IgD-CD27-		
Transitional B		
Plasmoblast		
DC		
mDC (CD11c+)		
pDC (CD123+)		
NK		
HLA-Dr+NK		
CD16+NK		
CD16-NK		
NKT (CD3+CD56+)		
Mono CD16 hi		
Mono CD16 lo		

SID	Sample	A/1	A/2	B/1	B/2	C/1	C/2	DPA#1	DPA#2	DPB#1	DPB#2	DQA#1	DQA#2	DQB#1	DQB#2	DRB#1	DRB#2	DRB345#1	DRB345#2
311GATCAGC	JM2_1055	A*02:01:01	A*33:01:01	B*14:02:01	B*44:05:01	C*02:02:02	C*08:02:01	DPA*01:03:01:01	DPA*02:01:01	DPB*04:01:01:01	DPB*14:01	DQA*03:01:01	DQA*05:05:01:01	-	-	DRB*04:02:01	DRB*11:04:01	DRB3*02:02:01:01	-
311TGTGATA	JM2_1088	A*01:01:01	A*24:02:01:01	B*08:01:01	B*52:01:01:01	C*07:01:01:01	C*12:02:02	DPA*01:03:01:01	DPA*02:01:01	DPB*04:01:01:01	DPB*14:01	DQA*01:03:01:01	DQA*05:01:01:01	DQB*02:01:01	DQB*06:01:03	DRB*03:01:01:01	DRB*15:02:01	DRB3*01:01:02:01	DRB5*01:02
311CGCGTGA	JM2_1121	A*02:01:01:01	A*29:02:01:01	B*44:02:01:01	B*44:03:01	C*05:01:01:01	C*16:01:01	DPA*01:03:01:01	DPA*02:02:02	DPB*01:01:01	DPB*04:02:01:01	DQA*01:01:01	-	DQB*02:02:01	DQB*05:01:01:01	DRB*01:01:01	DRB*07:01:01:01	-	-
311GCTGTAG	JM2_1193	A*03:01:01:01	-	B*07:02:01	B*57:02:01	C*04:01:01:01	C*07:02:01:01	DPA*02:01:01	-	DPB*01:01:02	DPB*27:01	DQA*01:02:01:01	DQA*01:01:02:02	DQB*02:02:01	DQB*06:02:01	DRB*07:01:01:01	DRB*15:01:01:01	DRB5*01:01:01	-
311ACAGCTG	JM2_1227	A*03:01:01:01	A*68:01:02	B*18:01:01	-	C*05:01:01:01	C*07:04:01	DPA*01:03:01:01	-	DPB*04:01:01:01	-	DQA*01:02:01:01	DQA*05:01:01:01	DQB*02:01:01	DQB*06:02:01	DRB*03:01:01:01	DRB*15:01:01:01	DRB3*02:02:01:01	DRB5*01:01:01
311ACAGCTG	JM2_1269	A*02:05:01	A*03:01:01:01	B*15:01:01	B*58:01:01	C*03:03:01	C*07:01:01:01	DPA*01:03:01:01	-	DPB*03:01:01	DPB*04:01:01:01	DQA*01:02:01:01	DQA*05:01:01:01	DQB*02:01:01	DQB*06:03:01	DRB*03:01:01:01	DRB*13:01:01	DRB3*02:02:01:01	-
311TGTACGC	JM2_1281	A*02:01:01:01	A*11:01:01	B*18:01:01	B*40:01:02	C*03:04:01:01	C*07:01:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*04:02:01:01	DQA*03:01:01	-	-	-	DRB*04:03:01	DRB*04:04:01	-	-
311AGTCATA	JM2_130	A*02:01:01:01	A*03:01:01:01	B*14:02:01	B*18:01:01:01	C*08:02:01	C*12:03:01:01	DPA*01:03:01:01	DPA*02:01:01	DPB*05:01:01	DPB*135:01	DQA*01:02:01:01	-	DQB*06:02:01	DQB*06:09	DRB*13:02:01	DRB*15:01:01:01	DRB3*03:01:01	DRB5*01:01:01
311TCACGCG	JM2_1373	A*11:01:01	A*26:02	B*27:04:01	B*40:01:02	C*03:03:01	C*08:01:01	DPA*02:01:01	DPA*02:02:02	DPB*02:02	DPB*107:01	DQA*03:02	DQA*05:05:01:01	-	-	DRB*09:01:02	DRB*12:01:01	DRB3*01:01:02:01	-
311CGCATCG	JM2_138	A*56:01	A*68:01:02	B*07:02:01	B*49:01:01	C*07:01:01:01	C*07:02:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*04:02:01:01	DQA*01:02:01:01	DQA*05:01:01:01	DQB*02:01:01	DQB*06:02:01	DRB*03:01:01:01	DRB*15:01:01:01	DRB3*01:01:02:01	DRB5*01:01:01
311TATGCGA	JM2_1459	A*11:01:01	A*24:02:01:01	B*08:01:01	B*36:03:01	C*02:02:02	C*04:01:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	-	DQA*01:02:01:01	DQA*05:01:01:01	DQB*02:01:01	DQB*06:02:01	DRB*03:01:01:01	DRB*15:01:01:01	DRB3*01:01:02:01	DRB5*01:01:01
311CAGCAGA	JM2_1485	A*01:01:01:01	A*02:01:01:01	B*08:01:01	B*49:01:01	C*07:01:01:01	-	DPA*01:03:01:01	DPA*02:02:02	DPB*04:02:01:01	DPB*05:01:01	DQA*01:02:01:01	DQA*05:01:01:01	DQB*02:01:01	DQB*06:02:01	DRB*03:01:01:01	DRB*15:01:01:01	DRB3*01:01:02:01	DRB5*01:01:01
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311GCTACTG	JM2_1625	A*01:01:01:01	A*02:01:01:01	B*44:02:01:01	B*45:01	C*05:01:01:01	C*16:01:01	DPA*01:03:01:01	DPA*02:01:01	DPB*04:02:01:01	DPB*05:01:01	DQA*03:01:01	DQA*04:01:01	-	-	DRB*04:02:01	DRB*08:02:01	-	-
311ACGCGAT	JM2_1632	A*02:01:01:01	A*23:01:01	B*15:16:01	B*44:05:01	C*02:02:02	C*14:02:01	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*105:01	DQA*03:01:01	DQA*05:01:01:01	-	-	DRB*03:02:01	DRB*04:01:01	DRB3*01:01:02:01	-
311TACTCTC	JM2_166	A*02:01:01:01	A*68:03:01	B*07:02:01	B*15:15	C*01:02:01	C*03:05	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*04:02:01:01	DQA*03:01:01	DQA*05:01:01:01	DQB*06:02:01	-	DRB*04:10:01	DRB*15:01:01:01	DRB3*01:01:01	-
311CGATCAT	JM2_1678	A*02:01:01:01	-	B*35:03:01	B*52:01:01:01	C*04:01:01:01	C*12:02:02	DPA*01:03:01:01	DPA*02:01:01	DPB*04:01:01:01	-	DQA*01:03:01:01	DQA*05:05:01:01	DQB*06:01:01	-	DRB*12:01:01	DRB*15:02:01	DRB3*02:02:01:01	DRB5*01:02
311GCTACGA	JM2_1680	A*02:01:01:01	A*11:01:01	B*15:01:01:01	B*51:01:01	C*03:03:01	C*14:02:01	DPA*01:03:01:01	-	DPB*02:01:02	DPB*04:01:01:01	DQA*03:01:01	DQA*05:05:01:01	-	-	DRB*04:01:01	DRB*11:01:01	DRB3*02:02:01:01	-
311TATCAGA	JM2_1706	A*02:01:01:01	A*33:03:01	B*07:02:01	B*58:01:01	C*03:02:02:01	C*07:02:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*26:01:02	DQA*01:02:01:01	DQA*01:03:01:01	DQB*06:01:01	DQB*06:09	DRB*13:02:01	DRB*15:02:01	DRB3*03:01:01	DRB5*01:02
311GAGCAGT	JM2_1739	A*01:01:01:01	A*23:01:01	B*08:01:01	B*57:01:01	C*06:02:01:01	C*07:01:01:01	DPA*02:01:02	-	DPB*01:01:01	DPB*107:01	DQA*03:01:01	DQA*05:01:01:01	DQB*02:01:01	-	DRB*03:01:01:01	DRB*04:02:01	DRB3*01:01:02:01	-
311AGACTAG	JM2_1761	A*01:01:01:01	A*30:02:01	B*08:01:01	B*51:01:01	C*07:01:01:01	C*16:01:01	DPA*02:01:02	-	DPB*01:01:01	-	DQA*05:01:01:01	-	DQB*02:01:01	DQB*02:02:01	DRB*03:01:01:01	DRB*07:01:01:01	DRB3*01:01:02:01	-
311TAGACTG	JM2_1766	A*02:01:01:01	-	B*08:01:01	B*44:02:01:01	C*05:01:01:01	C*07:01:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	-	DQA*01:02:01:01	-	DRB*04:01:01	DRB*15:01:01:01	DRB5*01:01:01	-	-	-
311TCGATC	JM2_1772	A*02:01:01:01	-	B*35:03:01	B*51:01:01	C*02:02:02	C*12:03:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*15:01	DQA*03:03:01	DQA*05:05:01:01	-	-	DRB*04:08:01	DRB*11:01:01	DRB3*02:02:01:01	-
311TGCTATC	JM2_1795	A*03:01:01:01	A*23:01:01	B*07:02:01	B*44:03:01	C*04:01:01:01	C*07:02:01:01	DPA*01:03:01:01	-	DPB*02:01:02	-	DQA*01:03:01:01	DQA*01:04:01:01	DQB*06:02:01	-	DRB*13:01:01	DRB*14:54:01	DRB3*02:02:01:01	-
311GCGCTCG	JM2_1827	A*01:01:01:01	A*02:01:01:01	B*08:01:01	B*55:01:01	C*03:03:01	C*07:01:01:01	DPA*01:03:01:01	-	DPB*02:01:02	DPB*04:01:01:01	DQA*01:04:01:01	DQA*05:01:01:01	DQB*06:03:01	DQB*06:03:01	DRB*03:01:01:01	DRB*14:54:01	DRB3*01:01:02:01	DRB3*02:02:01:01
311ATCATGC	JM2_1946	A*24:02:01:01	A*33:03:01	B*40:06:01:01	B*44:03:02	C*07:06	C*12:02:02	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*04:02:01:01	DQA*02:01	DQA*05:01:01:01	DQB*02:01:01	DQB*02:02:01	DRB*03:01:01:01	DRB*07:01:01:01	DRB3*02:02:01:01	-
311ATAGCAG	JM2_1966	A*02:01:01:01	A*24:02:01:01	B*07:02:01	B*36:03:01	C*04:01:01:01	C*07:02:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	-	DQA*01:02:01:01	-	DQB*06:02:01	DQB*06:04:01	DRB*13:02:01	DRB*15:01:01:01	DRB3*03:01:01	DRB5*01:01:01
311GATATGC	JM2_1997	A*03:01:01:01	A*11:01:01	B*13:02:01	B*27:02:01	C*02:02:02	C*06:02:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*104:01	DQA*05:05:01:01	-	DQB*02:02:01	-	DRB*07:01:01:01	DRB*11:01:01	DRB3*02:02:01:01	-
311CGTGCTA	JM2_201	A*30:02:01	A*68:01:02	B*40:01:02	B*44:03:01	C*03:03:04	C*03:04:01:01	DPA*01:03:01:01	-	DPB*01:01:01	DPB*04:01:01:01	DQA*04:01:01	DQA*05:05:01:01	-	-	DRB*08:02:01	DRB*08:06	-	-
311CACGATG	JM2_202	A*01:04N	A*11:01:01	B*15:01:01:01	B*35:01:01:01	C*04:01:01:01	-	DPA*01:03:01:01	DPA*02:01:01	DPB*03:01:01	DPB*03:01:01	DQA*01:01:01	DQA*01:03:01:01	DQB*06:04:01	-	DRB*01:01:01	DRB*13:01:01	DRB3*01:01:02:01	-
311CGTAGCG	JM2_223	A*24:02:01:01	A*24:07	B*35:05:01	B*39:01:01:01	C*04:01:01:01	C*07:02:01:01	DPA*02:02:02	-	DPB*05:01:01	DPB*135:01	DQA*01:04:01:01	DQA*05:01:01:01	DQB*02:01:01	DQB*05:02:01	DRB*03:01:01:01	DRB*14:54:01	DRB3*02:02:01:01	-
311GACTACA	JM2_251	A*01:01:01:01	A*30:01:01	B*13:02:01	-	C*06:02:01:01	-	DPA*01:03:01:01	-	DPB*105:01	-	DQA*05:05:01:01	-	DQB*02:02:01	-	DRB*07:01:01:01	DRB*13:05:01	DRB3*02:02:01:01	-
311CATGTAG	JM2_253	A*01:01:01:01	A*33:03:01	B*08:01:01	-	C*07:01:01:01	-	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*06:01	DQA*05:01:01:01	-	DQB*02:01:01	-	DRB*03:01:01:01	DRB*07:01:01:01	DRB3*01:01:02:01	-
311CTATACG	JM2_264	A*02:03:01	A*02:06:01	B*16:01:01	B*51:01:01	C*03:04:01:01	C*07:04:01	DPA*01:03:01:01	DPA*02:02:02	DPB*02:02	DPB*05:01:01	DQA*01:04:01:01	DQA*03:02	DQB*05:02:01	-	DRB*09:01:02	DRB*14:54:01	DRB3*02:02:01:01	DRB3*02:29N
311ACACACT	JM2_278	A*02:01:01:01	A*11:01:01	B*40:01:02	-	C*03:04:01:01	C*07:56:02	DPA*01:03:01:01	DPA*02:01:01	DPB*04:01:01:01	DPB*17:01	DQA*01:02:01:01	-	DQB*02:02:01	DQB*06:02:01	DRB*07:01:01:01	DRB*15:01:01:01	DRB5*01:01:01	-
311GATCGTA	JM2_314	A*68:01:02	-	B*15:18:01	-	C*07:04:01	-	DPA*02:01:01	-	DPB*04:01:01:01	DPB*14:01	DQA*01:03:01:01	DQA*01:04:02	DQB*06:01:01	DQB*06:03:01	DRB*13:01:01	DRB*14:04	DRB3*01:01:02:01	DRB3*02:02:01:01
311ACAGACG	JM2_321	A*01:01:01:01	A*26:01:01	B*08:01:01	B*35:01:01:01	C*07:02:01:01	-	DPA*01:03:01:01	-	DPB*04:01:01:01	-	DQA*05:01:01:01	DQA*05:05:01:01	DQB*02:01:01	-	DRB*03:01:01:01	DRB*11:01:01	DRB3*02:02:01:01	-
311ACGACGT	JM2_322	A*02:03:01	A*24:02:01:01	B*38:02:01	B*48:01:01	C*07:02:01:01	C*08:01:01	DPA*01:03:01:01	DPA*02:02:02	DPB*02:01:02	DPB*05:01:01	DQA*03:01:01	DQA*03:02	-	-	DRB*04:03:01	DRB*09:01:02	-	-
311GCATCTC	JM2_404	A*23:01:01	A*32:01:01	B*40:01:02	B*44:03:01	C*03:04:01:01	C*04:01:01:01	DPA*01:03:01:01	-	DPB*02:01:02	-	DQA*03:01:01	-	DQB*02:02:01	-	DRB*04:04:01	DRB*07:01:01:01	-	-
311TAGTGTG	JM2_411	A*02:01:01:01	A*24:02:01:01	B*44:02:01:01	B*44:05:01	C*02:02:02	C*05:01:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	DPB*04:02:01:01	DQA*01:02:01:01	DQA*05:05:01:01	DQB*06:02:01	-	DRB*12:01:01	DRB*15:01:01:01	DRB3*02:02:01:01	DRB5*01:01:01
311CGTACCG	JM2_427	A*25:01:01	A*68:01:01:01	B*27:05:02	B*35:03:01	C*02:02:02	C*04:01:01:01	DPA*01:03:01:01	-	DPB*04:01:01:01	-	DQA*01:02:01:01	DQA*05:01:01:01	DQB*02:01:01	DQB*06:02:01	DRB*03:01:01:01	DRB*15:01:01:01	DRB3*01:01:02:01	